

WHAT IS CLAIMED IS:

1. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein the peptide or fragment is effective in reducing HIV particle production, said method comprising:

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(a) introducing an expression construct into a mammalian cell or cells, wherein the expression constructs comprises a portion of the coding sequence for a mammalian *tsg101* gene;

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(b) introducing one or more expression constructs into the mammalian cell or cells, wherein the one or more expression constructs comprises the HIV *gag*, *pol*, and *rev* coding sequences;

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(c) incubating the transfected mammalian cell or cells in a suitable media for a sufficient time and at a temperature of about 37C to obtain a mammalian cell culture comprising mammalian cells which express a gene product encoded by the portion of a coding sequence for a mammalian *tsg101* gene and gene products encoded by the HIV *gag*, *pol* and *rev* genes;

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(d) measuring the level of particle associated p24 in the mammalian cell culture; and

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(e) correlating a reduced level of particle-associated p24, when compared to a control mammalian cell culture which has not been transfected with the expression construct comprising a portion of the coding sequence for a mammalian *tsg101* gene, with the identification of a peptide or fragment effective in reducing HIV particle production.

2. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein the peptide or fragment is effective in reducing HIV particle production, said method comprising:

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(a) introducing an expression construct into a mammalian cell or cells, wherein the expression construct comprises a portion of the coding sequence for a mammalian *tsg101* gene;

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(b) introducing HIV into the mammalian cell or cells;

(c) incubating the transfected and infected mammalian cell or cells in a suitable media for a sufficient time and at a temperature of about 37C to obtain a mammalian cell culture comprising mammalian cells which express a gene product encoded by the portion of a coding sequence for a mammalian *tsg101* gene and which produce HIV particles;

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(d) measuring the level of particle associated p24 in the mammalian cell culture; and

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(e) correlating a reduced level of particle-associated p24, when compared to a control mammalian cell culture which has not been transfected with the expression construct comprising a portion of the coding sequence for a mammalian *tsg101* gene, with the identification of a peptide or fragment effective in reducing HIV particle production.

3. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein the peptide or fragment is effective in reducing HIV particle production, said method comprising:

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(a) introducing an expression construct into a mammalian cell or cells, wherein the expression construct comprises a portion of the coding sequence for a mammalian *tsg101* gene;

(b) introducing HIV into the mammalian cell or cells;

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(c) incubating the transfected mammalian cell or cells in a suitable media for a sufficient time and at a temperature of about 37C to obtain a mammalian cell culture comprising mammalian cells which express a gene product encoded by the portion of a coding sequence for a mammalian *tsg101* gene and which produce HIV particles.

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(d) quantifying the HIV particles released from the cells; and

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(e) correlating a reduction in HIV particle production, when compared to a control mammalian cell culture which has not been transfected with the expression construct comprising a portion of the coding sequence for a mammalian *tsg101* gene, with the identification of a peptide or fragment effective in reducing HIV particle production.

4. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein the peptide or fragment is effective in reducing HIV particle production, said method comprising:

- 5 (a) introducing one or more expression constructs into the mammalian cell or cells, wherein the one or more expression constructs comprises the HIV *gag*, *pol*, and *rev* coding sequences;
- 10 (b) introducing into the mammalian cell or cells; a peptide or fragment derived from a mammalian Tsg101 protein;
- 15 (c) incubating the mammalian cell or cells transfected with the one or more expression constructs and permeated with a peptide or fragment derived from a mammalian Tsg101 peptide, in a suitable media for a sufficient time and at a temperature of about 37C to obtain a cell culture;
- 20 (d) measuring the level of particle associated p24 in the mammalian cell culture; and
- 25 (e) correlating a reduced level of particle-associated p24, when compared to a control mammalian cell culture which has not been permeated with a peptide or fragment derived from a mammalian Tsg101 protein, with the identification of a peptide or fragment effective in reducing HIV particle production.

5. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein the peptide or fragment is effective in reducing HIV particle production, said method comprising:

- 30 (a) introducing HIV into a mammalian cell or cells;
- 35 (b) introducing into the mammalian cell or cells; a peptide or fragment derived from a mammalian Tsg101 protein;
- 40 (c) incubating the mammalian cell or cells transfected with HIV and permeated with a peptide or fragment derived from a mammalian Tsg101 protein in a suitable media for a sufficient time and at a temperature of about 37C to obtain a cell culture;

- (d) measuring the level of particle associated p24 in the cell culture; and
- (e) correlating a reduced level of particle-associated p24, when compared to a control mammalian cell culture which has not been permeated with a peptide or fragment
5 derived from a mammalian Tsg101 protein, with the identification of a peptide or fragment effective in reducing HIV particle production.

6. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein the peptide or fragment is effective in reducing HIV particle
10 production, said method comprising:

- (a) introducing HIV into a mammalian cell or cells;
- (b) introducing into the mammalian cell or cells, a peptide or fragment derived from a
15 mammalian Tsg101 protein;
- (c) incubating the mammalian cell or cells transfected with HIV and permeated with a peptide or fragment derived from a mammalian Tsg101 protein in a suitable media and for a sufficient time, at a temperature of about 37C;
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- (d) quantifying the HIV particles released from the cells; and
- (e) correlating a reduction in HIV particle production, when compared to a control mammalian cell culture which has not been permeated with a peptide or fragment
25 derived from a mammalian Tsg101 protein, which the identification of a peptide or fragment effective in reducing HIV particle production.

7. The method of any one of claims 1, 2 or 3 wherein the coding sequence for a mammalian *tsg101* gene is from the UEV A domain of *Tsg101*.

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8. A peptide or fragment useful for reducing HIV particle production, said peptide comprising at least four contiguous amino acids of a mammalian Tsg101 protein and identified by the method of any one of claims 1-6.

9. The peptide or fragment of claim 8 wherein the mammalian Tsg101 protein is a mouse Tsg101 protein (SEQ ID NO:1), human Tsg101 protein (SEQ ID NO:2) or other mammalian Tsg101 protein.

5 10. The peptide or fragment of claim 8 wherein the four contiguous amino acids are from the UEV A domain of a mammalian Tsg101 protein.

11. The peptide of claim 8 wherein the peptide reduces HIV particle production by at least about three-fold.

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12. A pharmaceutical composition useful for reducing HIV particle production, said composition comprising one or more pharmaceutically acceptable carriers or diluents and a mammalian Tsg101 protein or a fragment thereof.

15 13. A pharmaceutical composition useful for reducing HIV particle production, which comprises one or more pharmaceutically acceptable carriers or diluents and a peptide according to claim 8.

14. The pharmaceutical composition of claim 13 wherein the Tsg101 protein is a
20 mouse Tsg101 protein (SEQ ID NO:1).

15. The pharmaceutical composition of claim 13 wherein the Tsg101 protein is a human Tsg101 protein (SEQ ID NO:2).

25 16. The pharmaceutical composition of claim 13 wherein the four contiguous amino acids are from the UEV A domain of a mammalian Tsg101 protein.

17. A method for identifying a peptide comprising the HIV-1 Gagp6 late (L) domain, PTAPP (SEQ ID NO:3), wherein the peptide inhibits interaction between a Tsg101
30 protein and HIV Gag, said method comprising:

(a) immobilizing on at least a first and second solid support or surface, a peptide comprising the HIV-1 Gag or HIV-1 Gagp6 late (L) domain, wherein the immobilized peptide is linked to a specific anti-Gag or L domain antibody or other linker;

(b) incubating at about 4C for a sufficient time, a first reaction mixture comprising labeled Tsg101 protein, a suitable buffer, and a peptide which comprises at least four contiguous amino acids of the HIV-1 Gagg6 late (L) domain;

5 (c) incubating at about 4C for a sufficient time, a second reaction mixture comprising labeled *tsg101* and a suitable buffer;

10 (d) adding the first reaction mixture of step (b) to the first solid support or surface mixture of step (a) and incubating in a liquid phase at a temperature of about 4C for a sufficient time;

15 (e) adding the second reaction mixture of step (c) to the second solid support or surface mixture of step (a) and incubating in a liquid phase at a temperature of about 4C for a sufficient time;

15 (f) separating the first solid support or surface from the liquid phase of step (d) to obtain a first solid phase;

20 (g) separating the second solid support or surface from the liquid phase of step (e) to obtain a second solid phase;

(h) determining the amount of labeled *tsg101* in the first solid phase of step (f) and in the second solid phase of step (g); and

25 (i) correlating a decrease in the amount of labeled *tsg101* in the first solid phase of step (f) when compared to the amount of labeled *tsg101* in the second solid phase of step (g) with the identification of a peptide comprising at least four contiguous amino acids of the HIV-1 Gagg6 late (L) domain wherein said peptide inhibits interaction between a Tsg101 protein and HIV Gag.

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18. The method of claim 17 wherein the Tsg101 protein is a mouse, human, or other mammalian Tsg101 protein.

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19. A peptide useful for inhibiting interaction between Tsg101 protein and HIV Gag, said peptide comprising at least four contiguous amino acids of the HIV-1 Gagp6 late (L) domain, PTAPP (SEQ ID NO:3) and identified by the method of claim 17.

5 20. The peptide of claim 19 further comprising at least five contiguous amino acids of ALQSRPEPTAPPEES (SEQ ID NO:4).

21. The peptide of claim 19 or 20 wherein the peptide causes at least about a three-fold reduction in TSG101-HIV Gag interaction.

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22. A pharmaceutical composition useful for inhibiting interaction between TSG101 and HIV Gag, said composition comprising one or more pharmaceutically acceptable carriers or diluents and a peptide according to claim 19 or 20.

15 23. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a peptide according to claim 8.

24. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a peptide according to claim 9.

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25. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a peptide according to claim 10.

25 26. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a peptide according to claim 11.

27. A method of treating a patient infected with HIV or other retrovirus which
30 comprises administering a therapeutically effective amount of a peptide according to claim 19 or 20.

28. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a pharmaceutical composition
35 according to claim 12.

29. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a pharmaceutical composition according to claim 13.

5 30. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a pharmaceutical composition according to claim 14.

10 31. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a pharmaceutical composition according to claim 15.

15 32. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a pharmaceutical composition according to claim 16.

20 33. A method of treating a patient infected with HIV or other retrovirus which comprises administering a therapeutically effective amount of a pharmaceutical composition according to claim 22.

25 34. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein said peptide or fragment is effective in reducing HIV particle production, said method comprising:

30 (a) measuring a level of HIV particles released in a culture of mammalian cells, wherein said mammalian cells comprise an expression construct comprising a portion of the coding sequence of a mammalian *tsg101* gene such that said mammalian cells express a gene product encoded by said portion of said coding sequence of said mammalian *tsg101* gene and are infected by HIV virus; and

35 (b) comparing said level of HIV particles to a level of HIV particles released in a culture of control mammalian cells under similar conditions, wherein said control mammalian cells do not comprise an expression construct comprising a portion of a coding sequence for a mammalian *tsg101* gene and are infected by HIV virus,

wherein said level of HIV particles measured in step (a) compared to said level of HIV particles of said culture of control mammalian cells below a predetermined threshold level identify said gene product encoded by said portion of said coding sequence of said mammalian *tsg101* gene as effective in reducing HIV particle production.

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35. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein said peptide or fragment is effective in reducing HIV particle production, said method comprising:

10 (a) measuring a level of HIV particles released in a culture of mammalian cells, wherein said mammalian cells comprise (i) an expression construct comprising a portion of the coding sequence of a mammalian *tsg101* gene and (ii) one or more expression constructs comprising the HIV *gag*, *pol*, and *rev* coding sequences such that said mammalian cells express a gene product encoded by said portion of said coding sequence of said mammalian 15 *tsg101* gene and gene products encoded by said HIV *gag*, *pol* and *rev* genes; and

(b) comparing said level of HIV particles released to a level of HIV particles released in a culture of control mammalian cells under similar conditions, wherein said control mammalian cells comprise one or more expression constructs comprising the HIV *gag*, *pol*, 20 and *rev* coding sequences such that said mammalian cells express gene products encoded by said HIV *gag*, *pol* and *rev* genes, and wherein said control mammalian cells do not comprise an expression construct comprising a portion of a coding sequence for a mammalian *tsg101* gene,

25 wherein said level of HIV particles measured in step (a) compared to said level of HIV particles released in said culture of control mammalian cells below a predetermined threshold level identify said gene product encoded by said portion of said coding sequence of said mammalian *tsg101* gene as effective in reducing HIV particle production.

30 36. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein said peptide or fragment is effective in reducing HIV particle production, said method comprising:

(a) measuring a level of HIV viral particle released in a culture of mammalian cells, 35 wherein said mammalian cells comprise (i) an expression construct comprising a portion of

the coding sequence of a mammalian *tsg101* gene and (ii) an expression construct comprising the HIV *gag* coding sequence such that said mammalian cells express a gene product encoded by said portion of said coding sequence of said mammalian *tsg101* gene and a gene product encoded by said HIV *gag* gene; and

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(b) comparing said level of HIV viral particle released to a level of HIV viral particle released in a culture of control mammalian cells under similar conditions, wherein said control mammalian cells comprise one or more expression constructs comprising said HIV *gag* coding sequence such that said mammalian cells express a gene product encoded by said 10 HIV *gag* gene, and wherein said control mammalian cells do not comprise an expression construct comprising a portion of a coding sequence for a mammalian *tsg101* gene,

wherein said level of HIV viral particle measured in step (a) compared to said level of HIV viral particle released in said culture of control mammalian cells below a predetermined 15 threshold level identify said gene product encoded by said portion of said coding sequence of said mammalian *tsg101* gene as effective in reducing HIV particle production.

37. The method of claim 34, 35, or 36, wherein said level of HIV particles released in a culture of mammalian cells and said level of HIV particles released in a culture of control 20 mammalian cells are represented by measured levels of particle associated p24.

38. The method of claim 34, 35, or 36, wherein said portion of said coding sequence of said mammalian *tsg101* gene is a sequence in the UEV A domain of said *Tsg101* gene.

25 39. The method of claim 38, wherein said predetermined threshold level is a two-fold reduction of said level of HIV particles measured in step (a) compared to said level of HIV particles of said culture of control mammalian cells.

40. The method of claim 38, wherein said predetermined threshold level is a four-fold 30 reduction of said level of HIV particles measured in step (a) compared to said level of HIV particles of said culture of control mammalian cells.

41. The method of claim 40, wherein said predetermined threshold level is a 90% reduction of said level of HIV particles measured in step (a) compared to said level of HIV 35 particles of said culture of control mammalian cells.

42. The method of claim 41, wherein said predetermined threshold level is a 95% reduction of said level of HIV particles measured in step (a) compared to said level of HIV particles of said culture of control mammalian cells.
- 5 43. The method of claim 42, wherein said predetermined threshold level is a 99% reduction of said level of HIV particles measured in step (a) compared to said level of HIV particles of said culture of control mammalian cells.
- 10 44. The method of claim 43, wherein said predetermined threshold level is a 99.5% reduction of said level of HIV particles measured in step (a) compared to said level of HIV particles of said culture of control mammalian cells.
- 15 45. A method for identifying a peptide or fragment derived from a mammalian Tsg101 protein, wherein said peptide or fragment is effective in reducing retrovirus production, said method comprising identifying a peptide that binds to a peptide comprising the PTAP motif of a retroviral protein.
- 20 46. The method of claim 45, wherein said retrovirus is a lentivirus.
- 25 47. The method of claim 46, wherein said lentivirus is HIV-1 virus.
48. The method of claim 46, wherein said lentivirus is HIV-2 virus.
49. The method of claim 46, wherein said lentivirus is simian immunodeficiency virus.
50. The method of claim 45, wherein said peptide comprising the PTAP motif is a HIV Gag protein.
- 30 51. The method of claim 45, wherein said peptide comprising the PTAP motif is the peptide of SEQ ID NO:4.
- 35 52. A method for identifying a peptide comprising a PTAP motif, wherein said peptide is effective in reducing retrovirus production, said method comprising identifying a peptide comprising a PTAP motif which binds to a TSG101 protein or a fragment thereof.

53. The method of claim 52, wherein said retrovirus is a lentivirus.

54. The method of claim 53, wherein said lentivirus is HIV-1 virus.

5 55. The method of claim 53, wherein said lentivirus is HIV-2 virus.

56. The method of claim 53, wherein said lentivirus is simian immunodeficiency virus.

10 57. The method of claim 52, wherein said peptide comprises the sequence of SEQ ID NO:4.

58. The method of any one of claims 34-36 and 45-57, wherein said tsg101 gene is a human tsg101 gene.

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